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Engineering Approaches Toward Deconstructing and Controlling the Stem Cell Environment

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Abstract

Stem cell-based therapeutics have become a vital component in tissue engineering and regenerative medicine. The microenvironment within which stem cells reside, *i.e.* the niche, plays a crucial role in regulating stem cell self-renewal and differentiation. However, current biological techniques lack the means to recapitulate the complexity of this microenvironment. Nano- and microengineered materials offer innovative methods to: (1) deconstruct the stem cell niche to understand the effects of individual elements; (2) construct complex tissue-like structures resembling the niche to better predict and control cellular processes; and (3) transplant stem cells or activate endogenous stem cell populations for regeneration of aged or diseased tissues. Here, we highlight some of the latest advances in this field and discuss future applications and directions of the use of nano- and microtechnologies for stem cell engineering.

Keywords

biomaterials; nano- and microfabrication; high-throughput; microfluidics; regenerative medicine

INTRODUCTION

What distinguishes stem cells from other cell types is the capability to self-renew and differentiate into lineage-specific progenies. These characteristics have made stem cells a promising tool in the fields of regenerative medicine[99] and cancer biology.[18] However, despite their potential, the translation from laboratory to clinic has been slow.[22] One reason is the inability to expand adult stem cells *in vitro* while preserving their differentiation capacity, and another is the lack of control over the differentiation of stem cells into desired cell types. To overcome these bottlenecks, it is crucial to understand the biology of stem cells and the molecular mechanisms governing stem cell self-renewal and lineage commitment.

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CONFLICTS OF INTEREST

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Common stem cell types include embryonic,[128] adult, and induced-pluripotent stem cells (iPSCs).[125] Adult stem cells have a limited differentiation capacity (multipotent), meaning they are able to form several lineages within a tissue and are organ specific. For instance, the bone marrow houses mesenchymal stem cells (MSCs) and hematopoietic stem cells (HSCs), whereas neural stem cells (NSCs) reside in the subventricular zone and hippocampus.[89] In contrast, embryonic stem cells (ESCs) and iPSCs are capable of producing all the cell types of an organism (pluripotent). ESCs are derived from the inner cell mass of the blastocyst of an embryo and iPSCs are generated by the genetic reprogramming of somatic cells into pluripotent stem cells.

It is becoming increasingly appreciated that the stem cell microenvironment, or niche, is responsible for regulating stem cell behavior and homeostasis.[43, 94, 95, 117, 132] Indeed, in their niche, stem cells are maintained or can undergo proliferation and differentiation in response to injury, disease, or aging to replenish lost cells or tissue. This homeostatic function is governed by intrinsic (genetic and epigenetic) as well as extrinsic (environmental) biological stimuli. The discovery of the niche and the continual uncovering of its constituents have allowed scientists to study the function of each component by deconstructing the niche into its individual parts.[11, 133] Recently, bioengineering methods have been instrumental in tackling biological questions that cannot be answered by conventional cell culture techniques.[20, 121] In this regard, engineering principles drawn from materials science to microfabrication have emerged to be useful, not only in the simplification but also the construction of an *in vitro* stem cell niche.[65]

The niche is composed of several constituents that work together to modulate stem cell function (Fig. 1). Inside this microenvironment, stem cells are exposed to a milieu of extracellular matrix (ECM), support or hub cells, and soluble factors. ECM is made up of proteins and polysaccharides that form a cross-linked network and impart structural and mechanical integrity to tissues. However, their role extends beyond acting as scaffolds to providing ligands that interact with cell receptors, such as integrins, to mediate cell adhesion, shape, migration, apoptosis, self-renewal, and differentiation.[80] Similarly, support cells interact with stem cells via membrane proteins. Soluble factors, such as cytokines, are another element that control stem cell behavior. Particular examples of such cytokines include wingless-related (Wnts)[109] and hedgehog proteins,[9] fibroblast growth factors (FGFs),[25] and bone morphogenetic proteins (BMPs).[143] Metabolic products, such as calcium, are another class of biological cues that affect stem cells. The impact of these biophysico-chemical components on the stem cell phenotype are an important design consideration in engineering the stem cell microenvironment, *in vitro*.

In this review article, recent progress in stem cell engineering is highlighted. Specifically, we dissect the stem cell niche into its individual elements including scaffold, biophysical and biochemical factors, and describe how engineering approaches are being applied towards studying these elements. Furthermore, recent technologies that have been used or are promising for the fabrication of complex, tissue-like structures using stem cells are described. We conclude by introducing the role of advanced biomaterials in stem cell-based *in vivo* therapeutics.

CONSTRUCTION OF A BIOMIMETIC ARTIFICIAL NICHE

The traditional 2-dimensional (2D) culture system has afforded experimenters with simplicity in the analysis of individual variables affecting cells. However, this simplification has led to a disjoint in the translation of *in vitro* results to *in vivo* systems. A 3-dimensional (3D) platform, while more complex, better mimics the *in vivo* organization of cells and is a crucial requirement for tissue construction.[11, 105] The first step in this process is the

selection of the appropriate material as the cellular scaffold. Currently, *in vitro* cell cultures often utilize rigid polystyrene surfaces or extracted ECM proteins, such as collagen or laminin. Also, the use of animal-based feeder layers is common in ESC cultures. These culture techniques lack 3D spatial complexity, are not amenable to chemical or physical modification, and employ xenogeneic cells. To overcome these impediments, advanced materials can be designed to incorporate cells and present biochemical signals, such as adhesive ligands and cytokines. Furthermore, mechanical properties, such as porosity, stiffness, and degradation are tunable. For detailed review of biomaterials in stem cell biology and tissue engineering, we refer the readers to several excellent reviews.[24, 29, 82, 86, 115] As more discoveries are made regarding the role of biophysical and biochemical factors in stem cell regulation, the design of materials that incorporate these findings will enable more control over the fate of these cells.

BIOMATERIALS AS STRUCTURAL FRAMEWORKS FOR STEM CELLS

Biomaterial-based scaffolds serve as a framework for a 3D stem cell niche *in vitro*. These materials will function as platforms for cell attachment, migration, proliferation and differentiation. In designing these materials, criteria that must be considered include biocompatibility, fluid transport for the exchange of gases and nutrients, and biochemical and mechanical integrity for cellular processes.

Biomaterials can have a variety of structures depending on their composition and processing methods. Hydrogels are one type of structure that can be fabricated, and this type of biomaterials has wide utility in stem cell biology. Hydrogels are a 3D network of cross-linked hydrophilic polymers (natural or synthetic) containing 95–99% water, whose physico-chemical properties are highly tunable.[135] This class of materials is a particularly attractive cellular scaffold substitute given its inherent structural similarity to ECM.[45, 122] Hydrogels are synthesized by the presence of a cross-linking agent through numerous schemes, such as free radical polymerization,[134] Michael addition,[83] or click chemistry.[85] Microfabrication techniques have been utilized to cultivate hydrogels with desired microarchitectures. These techniques include emulsification,[96] micromolding,[140] photocrosslinking,[53, 70] microfluidics,[10, 101] and bioprinting.[91] Besides hydrogels, other structures include fibrous[6] or macroporous[68] scaffolds, often formed through electrospinning or porogen leaching, respectively (Fig. 2a).

Nature-Derived Biomaterials

The material used for 3D stem cell culture can be naturally-derived or synthetic. Natural biomaterials are often acquired from isolating ECM components, such as collagen, hyaluronic acid (HA), and fibronectin, but are also derived from non-ECM materials, such as chitosan and silk.[24] Gerecht *et al.*[47] used chemically modified HA to form photopolymerizable hydrogels for human ESC encapsulation. Human ESCs encapsulated in these hydrogels maintained their pluripotency, and upon exposure to vascular endothelial growth factor (VEGF) differentiated to the vascular lineage. Recently, decellularized organs have also been used as scaffolds to generate tissue-engineered lung,[106] liver,[129] heart,[100] and bone.[50] For instance, bone grafts in the shape of the temporomandibular joint condylar bone were engineered using a bioreactor and decellularized trabecular bone seeded with human MSCs (Fig. 2b).[50] The advantages of natural scaffolds are their biocompatibility and provision of biological cues, however batch-to-batch variability, potential adverse immunogenicity and poor control over physico-chemical properties limits the use of these materials.

Synthetic Biomaterials

Synthetic materials have come to fill the gap created by their natural counterparts due to greater control over their mechanical and chemical properties. The most widely used synthetic materials in stem cell engineering are polymers, particularly polyacrylamides, polyacrylates, polyethers and polyesters.[86, 115] These scaffolds can be modified to include adhesion molecules, such as arginine-glycine-aspartate (RGD), which is a ubiquitous, integrin-binding domain of fibronectin[58]; additionally, growth factors[36] and protease-sensitive peptide sequences for scaffold degradation can be incorporated into the scaffold (Fig. 2c).[104] In designing these scaffolds, biodegradability is an important criteria under consideration; the rate of degradation ideally should be comparable to the rate of ECM deposition and tissue formation by the cells seeded within the scaffold. Polyesters, such as poly (glycolic acid) and poly(L-lactic acid) (PLLA), have long been used in biological settings, given their broad range of degradability, and that their degradation byproducts can be cleared through endogenous metabolic pathways.[24] For instance, poly(lactic-*co*-glycolic acid)/PLLA copolymeric scaffolds have been used for human ESC differentiation and formation of structures resembling neural, cartilage, or liver tissues in the presence of various growth factors.[76] In contrast to polyesters, poly(ethylene glycol) (PEG) does not biodegrade; one way of making this class of synthetic material biodegradable is through using a peptide cross-linker sensitive to proteases during the polymerization of PEG units.[84] Degradation occurs as cells in the scaffold secrete proteases, such as matrix metalloproteases, cleaving the peptide cross-linkers. In addition, given the inert nature of PEG, it can be used as a base scaffold to present small organic molecules, peptides and proteins. For instance, using poly(ethylene glycol) (PEG) hydrogels with tethered small chemical functional groups, human MSCs were induced to differentiate towards osteogenic and adipogenic lineages.[7] Interestingly, charged phosphate functional groups, prominent in mineralized bone, were responsible for osteogenic induction, whereas hydrophobic groups, reflecting the lipid environment of adipose tissue, resulted in adipogenic differentiation.

Synthetic biomaterials and microfabrication technologies have been combined to generate homogeneous populations of stem cells.[59, 62, 92, 126] Cross-linkable polymers and photolithography can be used to construct PEG hydrogel microwells to generate a uniform array of controlled-size embryoid bodies (EBs).[62, 92, 126] EBs are multicellular aggregates of ESCs that recapitulate the early steps in development. Hwang *et al.*[59] used such a platform to control EB size based on the microwell dimension, and study its effects on ESC differentiation. Smaller EBs favored vascular differentiation mediated via expression of Wnt5a, whereas larger EBs promoted cardiomyogenesis through expression of Wnt11. Photolithography can also be used to create asymmetrical microenvironments to recapitulate *in vivo* developmental processes. Qi *et al.*[108] encapsulated one-half of an EB in PEG hydrogel and the other half in gelatin methacrylate hydrogel. This resulted in polarization of EBs and vasculogenic differentiation in a spatially dependent manner.

In addition to polymers, inorganic materials, such as ceramics[142] and metals[78] have also been under investigation, primarily as osteogenic materials in MSC differentiation.

Creating Biomaterial Libraries

Given the vast number of ECM and synthetic molecules used as scaffolds and the difficulty in predicting the reaction of stem cells to such molecules, high-throughput screening is needed for an efficient and cost-effective method of creating material libraries.[37, 69] Such screening can be done with the aid of robotically-assisted, nanoliter-scale liquid dispensing, and printing methods. Inone example, Flaim *et al.*[38] created a 32 ECM combinatorial platform made from five ECM molecules (collagen I, III, IV, laminin, and fibronectin) and demonstrated that hepatocyte differentiation from mouse ESCs was enhanced ~140-fold

between the least and the most effective combinations. In another work, arrays of self-assembled monolayers presenting varying peptide sequences and densities were used to screen for ESC growth and maintenance.[26] An appropriate peptide sequence was chosen based on the screening results, and used to create a 3D hydrogel scaffold that, as expected, promoted ESC self-renewal. High-throughput platforms have also been applied to analyze the effects of combinatorial libraries of synthetic polymers on stem cells. For instance, Anderson *et al.*[5] tested over 1,700 human ESC-acrylate-based polymer interactions using nanoliter quantities of materials to measure cellular attachment, proliferation, and differentiation (Fig. 2d). Using their microarray, materials that enhanced cell attachment, growth, and differentiation into cytokeratin-positive cells were identified.

BIOPHYSICAL ASPECTS OF THE STEM CELL NICHE

ECM interaction with cells, mediated through cell membrane receptors, is both biochemical and biophysical in nature. The biophysical features experienced by stem cells include topography, matrix stiffness, and dynamic forces. These physical cues exerted on the cell by the microenvironment are transduced to biochemical intracellular signals and cytoskeletal tension through actomyosin contractility that result in cellular proliferation, apoptosis, adhesion, migration, or differentiation.[40, 138] These interactions serve as a salient regulator of stem cell fate.[30, 52]

Topography

ECM is known to exhibit topographical cues at the nano- and micrometer scales that interact with cells. For instance, collagen and elastin have a fibrillar structure with nanometer dimensions. This interaction with cells is through a phenomenon known as contact guidance. Contact guidance is the process by which ECM provides directional cues that will determine cell morphology and migration.[111] Nanoscale technologies have been used to create surface geometrical arrays of nanoposts, nanogrooves, and nanopits.[8, 33, 71, 124, 136] Methods used to fabricate these topographical cues include traditional nanomolding, photolithography, electron-beam,[131] and dip pen[75] lithography.[97] These arrays have been shown to induce cytoskeletal changes and commitment in stem cells. For instance, fibronectin-coated poly(dimethylsiloxane) (PDMS) nanometer line gratings resulted in alignment and elongation of human ESCs through the organization of actin, vimentin, and α-tubulin.[46] Similarly, in work by Yim *et al.*[141], human MSCs that were cultured on collagen-coated PDMS nanogratings showed increased cytoskeletal and nuclei alignment, as well as upregulation of microtubule-associated protein 2, a neuronal marker, compared to unpatterned and micropatterned controls (Fig. 3a). In work by Oh *et al.*[98], human MSC differentiation was controlled by altering the size of the titanium oxide nanotubes on which cells were cultured. Notably, small diameter (~30-nm) nanotubes maintained multipotency while larger (~70- to 100-nm) nanotubes promoted differentiation to osteoblast-like cells in the absence of osteogenic inducing factors. In another work, Dalby *et al.*[21] showed that human MSCs grown on slightly disordered polymethylmethacrylate nanopits resulted in the increased production of bone-specific extracellular matrices, osteopontin and osteocalcin, in the absence of osteogenic inducing media. There has not been a clear consensus on the mechanism explaining the observed cellular phenotypic changes due to topography. One theory states that such changes originate from the generation of anisotropic stresses that may be due to clustering of focal adhesions or directional actin polymerization.[8] Although the exact mechanism is yet to be determined, topographical features add another degree of control over scaffold design for stem cell engineering.

Microarchitecture and Cell Shape

During embryonic development, cells change their shape as they undergo differentiation. Indeed, cell shape is known to affect proliferation,[41] apoptosis,[15] nuclear organization,[73] and differentiation.[44, 66, 88] There is an intricate link between cell shape and cell-ECM interaction, which is mediated through cytoskeletal changes.[35] The local microarchitecture and geometry of the niche, imparted by ECM and neighboring cells, is one of the physical cues experienced by stem cells. Microfabrication techniques have allowed for the manipulation of cell shape, through creating cell-adhesive geometric patterns that in turn lead to changes in cell function.[65] For example, McBeath *et al.*[88] demonstrated that lineage commitment of stem cells can be controlled through cell morphology. In their study, human MSCs were grown on adhesive substrates that either allowed flat or round morphologies. Osteogenesis was favored in cells that adhered and spread, whereas adipogenesis was enhanced in cells that did not spread. Furthermore, these commitments were dependent on RhoA activity, known to be involved in cytoskeletal remodeling, and actomyosin tension. Using the same patterned substrates, human MSCs were selectively differentiated to myogenic (flattened morphology) or chondrogenic (round shape) fates in the presence of transforming growth factor β.[44]

To further elucidate the interwoven roles of cytoskeletal tension and cell shape on stem cell fate, in another study, geometrical patterns of varying aspect ratios and subcellular curvatures were tested on human MSCs (Fig. 3b).[66] Shapes that had higher aspect ratios and greater curvatures promoted increased contractility and preferential osteogenesis, whereas shapes resulting in low contractility favored adipogenesis. The effect of cytoskeletal contractility on stem cell fate, mediated through cell shape, was confirmed by the use of cytoskeletal-disrupting agents.

The previous examples demonstrate that human MSCs can be, depending on the growth factors present, directed to osteogenic[66, 88] and myogenic[44] fates when cell spreading and increased contractility are favored, whereas adipogenic[66, 88] and chondrogenic[44] differentiation are enhanced when cell size and contractility are reduced. Further, these studies demonstrate the delicate interplay between cytoskeletal rearrangement, cell shape, and differentiation. Such an interplay may be explained by (1) changing cytoskeletal arrangement leading to a distorted nucleus;[119] and (2) focal adhesion assembly induction.[14]

Substrate Stiffness and Dynamic Forces

Not only can the ECM influence the behavior of stem cells through its topographical/geometric features, its inherent mechanical properties add another dimension to the complexity of the stem cell niche and control over stem cell regulation. Materials science and polymer chemistry have been used to fabricate materials with tunable stiffness. One common method is by altering the degree of cross-linking of hydrogels.[122] In a seminal work, Engler *et al.*[34] used collagen I-coated polyacrylamide gels with elastic moduli resembling brain, muscle and bone tissues to induce the commitment of human MSCs into neurogenic, myogenic and osteogenic lineages, respectively. In the previous example, cells were grown in a 2D environment. To study the effects of matrix stiffness on stem cell fate in a more physiologically relevant environment, MSCs were encapsulated in 3D RGD-modified alginate hydrogels of varying elastic moduli.[57] Osteogenesis was predominant in hydrogels with elastic moduli of 11–30 kPa, whereas adipogenesis occurred more in softer gels (2.5–5 kPa). While the aforementioned studies control adhesive ligand densities in their materials, other material properties, such as surface chemistry and porosity, cannot be effectively controlled in the fabrication process of materials with varying stiffness. To isolate the effect of substrate rigidity, PDMS microposts of different rigidities were fabricated by varying the post's height (Figure 3c).[42] Human MSCs grown on rigid (short)

microposts favored osteogenesis whereas adipogenesis occurred on softer (tall) microposts. Substrate elasticity can also be used to direct the fate of other stem cells, such as hematopoietic,[56] neural,[114] and muscle.[48]

Whereas stem cell fate can be dictated by static forces through matrix stiffness, during organogenesis as well as adult life, stem cells are exposed to dynamic forces that also affect their function.[1, 16] For instance, local cyclic stress applied through magnetic twisting cytometry to single ESCs resulted in the downregulation of pluripotency genes, Oct3/4, as compared to unexposed cells in the same culture dish.[16] Moreover, shear forces from flowing fluids have been found to induce endothelial[139] and hematopoietic lineage differentiation. For example, Adamo *et al.*[1] described the preferential differentiation of mouse ESCs into hematopoietic progenitor cells when exposed to shear stress as opposed to static culture. Briefly, mouse ESCs, cultured on 1% gelatin-coated culture plates, were exposed to a step-wise increase in shear stress from 0–5 dyne per cm^2, followed by a constant 5 dyne per cm^2. Compared to static culture, the cells in dynamic culture had a higher expression of the endothelial and hematopoietic marker, CD31 (PECAM1).

BIOCHEMICAL PARAMETERS AFFECTING STEM CELLS

In addition to the cellular scaffold and physical facets, biochemical factors are a vital component of the stem cell niche. These biochemical factors present themselves in a dynamic, spatiotemporal manner that is difficult to mimic in standard *in vitro* culture systems. Identifying relevant biomolecules involved in stem cell modulation is critical to understanding mechanisms of self-renewal and differentiation, as well as directing such processes.

Static Biochemical Presentation

In the stem cell niche, cytokines and other biochemical signals are released by stem cells (autocrine), support (paracrine), or distant cells (endocrine) that act on stem cells to induce functional changes. Cytokines can be incorporated into biomaterial scaffolds in soluble (interspersed), immobilized, or encapsulated forms. One of the most common methods of assimilating cytokines into scaffolds is by dispersing them in the interstices of the material. For instance, BMP-2 and human MSCs were added prior to gelation, and encapsulated in HA hydrogels to induce osteogenesis in rat calvarial defects.[67] Hydrogels with human MSCs and BMP-2 had the most enhanced mature bone formation with vascular markers being present, compared with hydrogel alone, hydrogel and MSCs, or hydrogel and BMP-2.

While cytokines are part of the culture media in conventional culture systems, *in vivo*, they are often tethered to the ECM. To replicate this mode of presentation, cytokines or specific biologically-active sequences of cytokines (peptides) have been immobilized onto biomaterial scaffolds. For example, Alberti *et al.*[3] immobilized leukemia inhibitory factor (LIF), conventionally used in ESC culture to inhibit differentiation, to maleic anhydride copolymer thin-film coatings, and showed that stem cell pluripotency could be maintained for at least two weeks in the absence of soluble LIF. Covalent bonding of proteins to scaffolds may undoubtedly raise concern over the structure and bio-functionality of the tethered protein. To overcome this concern, other biomimetic methods have been developed. Glycosaminoglycans (GAGs), as part of proteoglycans (a component of ECM), are known to bind growth factors via electrostatic interactions, leading to their sequestration and reduced degradation.[107] Heparin, a well-known GAG, has been incorporated in scaffolds to immobilize growth factors and influence stem cells.[103] Another method of incorporating cytokines is by encapsulating them into polymer-based nano- or microspheres embedded within scaffolds.[54, 110] For instance, biodegradable poly(lactic-*co*-glycolic acid) microspheres loaded with retinoic acid (RA) were fabricated by water-in-oil single emulsion

and incorporated in EBs.[13] In EBs containing RA-loaded microspheres, ESC differentiation was more organized and better resembled early embryogenesis compared to EBs exposed to soluble RA in culture media. These delivery particles allowed for a controlled release and protected the biomolecule from inactivation.[107]

Miniaturized high-throughput platforms have also been extended to efficiently study stem cell-cytokine interactions. These assays have been employed to identify peptides[26] and screen for small molecules,[28] and combinations of ECM proteins and growth factors.[39, 72, 123] For example, an array of combinations of ECM components and cytokines were printed onto a surface and seeded with human neural precursor cells. Self-renewal and proliferation were enhanced by the co-stimulation of Wnt and Notch, whereas BMP-4 induced differentiation into cells expressing neural and glial markers. These microarrays can be used to discover new interactions between stem cells and their soluble environment for the development of new pharmaceutical therapeutics. In addition, the large quantity of data obtained via these methods poses a challenge and necessitates a need for an accurate, efficient and automated computer-aided analysis.

Dynamic Biochemical Presentation

The stem cell niche *in vivo*, unlike conventional culture systems, is a dynamic milieu where biochemical cues are presented in a spatially and temporally distinct manner. The use of microfluidics has added valuable insight and allowed for generation of a more dynamic microenvironment for stem cell manipulation. Microfluidics is the science of manipulating fluids using channels with dimensions in the micrometer range.[137] Currently, the most popular material used to fabricate microfluidic devices is PDMS, which is well suited for biological applications given its low toxicity, high transparency, and permeability to oxygen and carbon dioxide.[130] Given that flow is laminar in such devices, mixing occurs via diffusion; hence, there is great control over the spatial and temporal concentrations of molecules (Fig. 4a). There are numerous methods of generating chemical concentration gradients that have been reviewed in the literature.[63, 116]

The presentation of numerous soluble factors (*e.g.* cytokines and oxygen) and physical parameters (*e.g.* shear stress and temperature) can be controlled with microfluidics. By exposing a *Drosophila melanogaster* embryo to a temperature gradient along its anterior-posterior axis, Lucchetta *et al.*[79] were able to generate a differential cellular proliferation within the embryo along the temperature gradient. The generation of chemical gradients via diffusive mixing, devised by Dertinger, Jeon, and co-workers, has been used extensively to study chemotaxis and other biological phenomena involving gradients.[27, 60] Using a tree-like gradient-generating microfluidic system, Chung *et al.*[17] studied the effects of a concentration gradient of a mixture of epidermal growth factor, FGF-2, and platelet-derived growth factor on the differentiation of human NSCs into astrocytes. One of the limitations of microfluidics has been the adverse effect of the shear stress on cells. Other methods of generating gradients, such as using an osmotic pump, have been introduced to reduce the magnitude of the shear stress produced. For instance, Park *et al.*[102] used an osmotic pump to generate continuous gradients of cytokines (sonic hedgehog, FGF-8, and BMP-4) to direct the differentiation of ESC-derived neural progenitors into neurons.

Analysis of *in vitro* cultures of mixed cell colonies results in the average response of the entire population, masking the responses of individual or rare cells. Hence, there is a need for single cell culture and analysis platforms. Microfluidics have generated approaches to address this issue. Lecault *et al.*[74] fabricated a multi-layered microfluidic device with nanoliter-size chambers and automated medium exchange to study HSC proliferation in response to Steel factor (Fig. 4b). Such a device allows for single-cell level analysis,

continuous medium exchange, parallel studies of temporally-varied exposure of cells to cytokines (in this case, Steel factor), and time-lapse imaging.

COMPLEX TISSUE CONSTRUCTION

Thus far, we have broken down the stem cell niche into its individual elements to better comprehend the role of each component in stem cell function. However, in order to materialize the potential benefits of stem cell engineering-driven therapeutics, construction of complex, hierarchical structures and tissues is necessary. Tissue printing is a promising technology for such 3D tissue fabrication.[90, 91] Using a 'bottom-up' approach, tissue printing uses robotically-driven, layer-by-layer deposition of cells and matrices without the use of a scaffold (Fig. 5a).[12] Although this technology has not been utilized widely in stem cell biology, it has the potential to make an impact. For instance, bioprinting has been used to create distinct spatial patterns of immobilized BMP-2 and FGF-2 that resulted in the commitment of primary muscle-derived stem cells to osteoblast and tenocyte lineages, respectively, when on-pattern and myogenic lineage when off-pattern.[64]

Another technology of interest is electropatterning for the construction of 3D microenvironments. Albrecht *et al.*[4] used dielectrophoretic forces to form multicellular structures ranging from a few to >20,000 cell clusters in photopolymerizable hydrogels and demonstrated that chondrocyte synthesis of sulfated GAG was dependent upon cluster size (Fig. 5b). Another 'bottom-up' approach is cell-laden hydrogel modular assembly.[61] In this method individual building blocks or microstructures of a tissue are built and assembled into a cohesive tissue-like structure (Fig. 5c). Each unit may contain a particular cell type and embody a scaffold with unique mechanical and biochemical properties. In this way, not only is the architecture of the desired tissue defined, but also the cellular arrangements can be meticulously delineated. In one example, Du *et al.*[31] assembled tubular structures of concentric hydrogel constructs with an inner layer of encapsulated endothelial cells and outer layer of smooth muscle cells, emulating native arterial cellular organization.

IN VIVO APPLICATIONS OF STEM CELL ENGINEERING

Up until now, we have discussed the application of biomaterials for the *in vitro* study of stem cell biology. Biomaterials can also be used as delivery vehicles for stem cells *in vivo* for the repair of damaged or degenerated tissue.[30, 81, 82, 93] Currently, most cell-based therapies involve injecting cells in a liquid medium into the site of interest. However, in this method of delivery, cells have low survivability due to anoikis, poor engraftment, and in the case of stem cells, control over differentiation is lacking.[93] Advanced biomaterials have great potential in overcoming these shortcomings. These materials must be designed to create an environment for cell survival, provide support cells that function to enhance the endogenous stem cell population, and/or present regulatory cues that will modulate delivered or endogenous stem cells. Using this strategy, Hill *et al.*[55] transplanted alginate scaffolds containing immobilized RGD, soluble hepatocyte growth factor and FGF-2, and satellite cells to damaged mouse muscle tissue. This resulted in enhanced activation and migration of transplanted cells and repopulation of damaged muscle tissue as compared with simply injecting satellite cells. In another study, Silva *et al.*[120] showed that alginate scaffolds containing RGD and VEGF along with a combination of outgrowth endothelial cells and endothelial progenitor cells can salvage ischemic murine limbs and restore limb perfusion as compared to dual cell injection (Fig. 6).

While stem cell transplantation remains a viable option in tissue repair, therapeutic targeting of endogenous stem cell niches has been achieved via soluble cues and support cells.[2, 19, 113] Smart biomaterials can be applied to present localized, bioactive molecular cues or support cells targeting specific stem cell niches *in vivo*.[51, 112, 144] These molecular cues may include

growth factors and morphogens that can be released in a controlled fashion and target desired niches to affect the proliferation and differentiation of cells. For instance, Gomi *et al.*[49] were able to drive heterotopic hematopoiesis of host origin and osteogenesis in a subcutaneously implanted polyester scaffold seeded with osteogenic cells. Furthermore, by injecting self-assembling peptide nanofibers into the myocardium, Davis *et al.*[23] showed vascular progenitor cell recruitment.

CONCLUSIONS AND FUTURE PROSPECTS

Undoubtedly, stem cell-based therapeutics have become a tantalizing approach to the problems facing regenerative medicine. Aging, disease, and trauma can often lead to loss of tissues and with it, its function necessary for a patient's quality of living and survival. Given their differentiation capacity, stem cells are an attractive choice to replace lost tissues. Since isolation of human ESCs and genetic engineering of iPSCs have occurred only in recent history, the merger of stem cell biology and engineering approaches are still nascent. Current conventional approaches to stem cell biology employ materials and systems that do not appropriately capture the spatial and temporal biochemical and biophysical cues present *in vivo*. This has resulted in frustration over controlling stem cell fate and translating *in vitro* work to animal models and human clinical trials. Microengineering and materials science disciplines have come to illuminate the complexity of the stem cell niche through novel, physiologically-relevant culture platforms. Such mimicry through multiplexed systems is necessary to study stem cell biology and pave the way towards clinical relevance. While nano- and microtechnologies have enabled precise control over studying the effects of individual components of the stem cell niche, the integration of these different elements in tissue engineered constructs will be the focus of future research.

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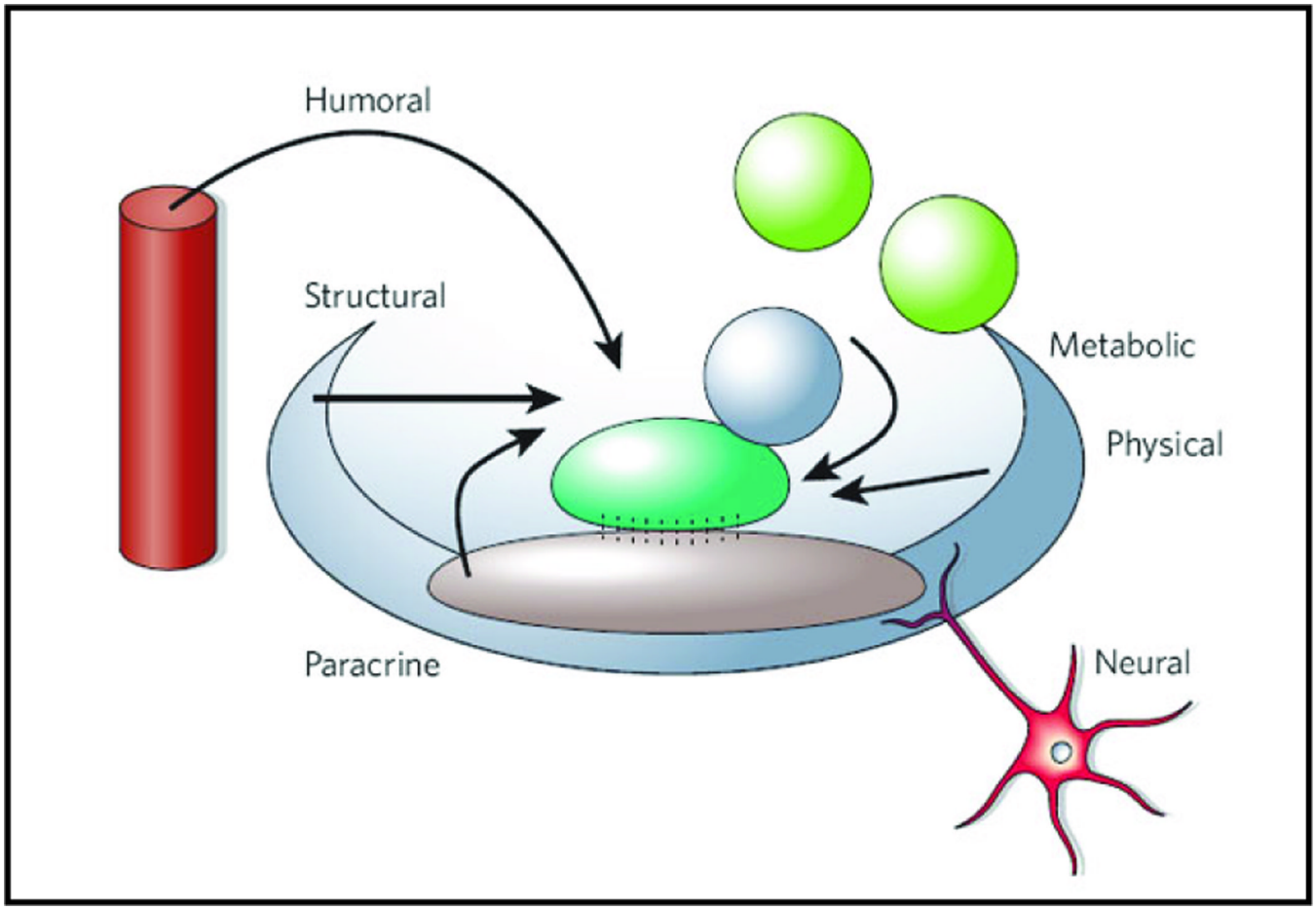

Figure 1.
The stem cell microenvironment, or niche. In the niche, stem cells are exposed to ECM, soluble and immobilized molecules (cytokines, metabolic products), support cells, and physical cues (topography, structure, stiffness, static and dynamic forces). Stem cell is represented in dark green and progenitor cell in light gray.[117] Reprinted by permission from Macmillan Publishers Ltd: [Nature], copyright (2006).

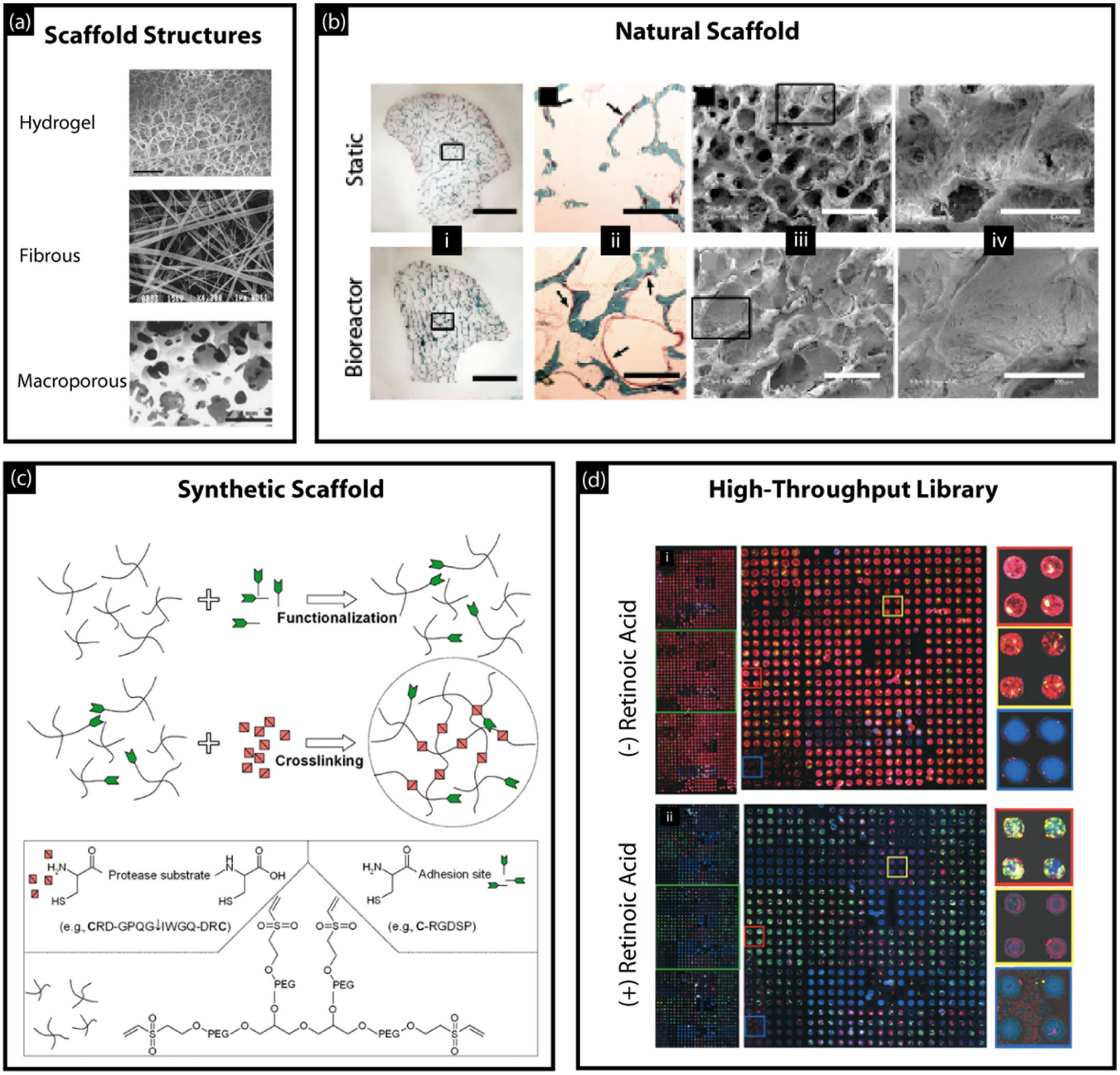

Figure 2.

Engineered scaffolds for stem cell culture. (a) Scaffolds can be designed to have hydrogel,[77] (Adapted with permission from Springer Science) fibrous,[87] (Adapted with permission from American Chemical Society)or macroporous[118] (Copyright (2000) National Academy of Sciences, USA.) structures. (b) Decellularized bone seeded with human MSCs demonstrated enhanced bone formation by perfusion. (i and ii) Osteoid formation demonstrated by trichrome staining showing new (red) and old (green) matrix. (iii and iv) scanning electron micrographs showing confluent layers of lamellar bone.[50] Scale bars: (bi)=5 mm, (bii) and (biii)=1 mm, (biv)=500 μm. Copyright (2010) National Academy of Sciences, USA. (c) Functionalization of PEG with cell adhesion ligand, RGD and protease-sensitive peptide sequence.[84] Reprinted by permission from Macmillan Publishers Ltd: [Nature

Biotechnology], copyright (2003). (d) Human ESCs seeded on synthetic polymer arrays, stained for cytokeratin (green) and vimentin (red) with or without exposure to retinoic acid.[5] Adapted with permission from Macmillan Publishers Ltd: [Nature Biotechnology], copyright (2004).

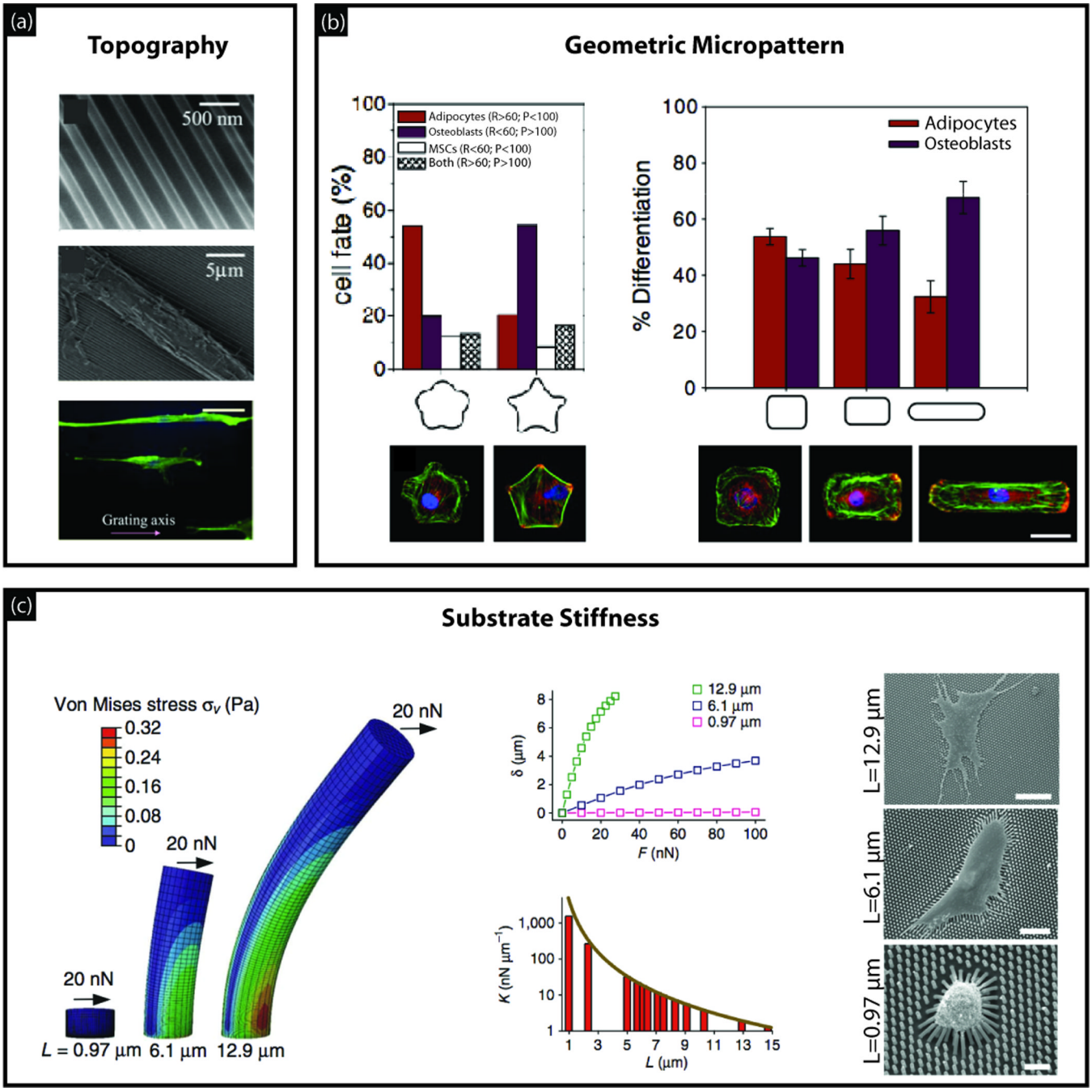

Figure 3.

Role of physical cues on stem cells. (a) Scanning electron micrographs of PDMS nano-patterns (top) and human MSCs on a nano-patterned substrate (middle) and F-actin immunostaining (bottom).[141] Adapted with permission from Elsevier. (b) Cell shape directing MSC differentiation.[66] Osteogenesis was enhanced on micropatterns of high aspect ratio and patterns with sharp edges, whereas adipogenesis was favored on low aspect ratio patterns and those with curved edges. Immunofluorescent images of cells stained for F-actin (green), vinculin (red) and nuclei (blue). Scale bar: 30µm. Copyright (2010) National Academy of Sciences, USA. (c) PDMS microposts were fabricated with differing rigidities by changing post's height.[42] Rigidity was characterized by computing nominal spring

constant, *K*. MSC commitment was differentially directed using these microposts. Scale bars: top=50μm, middle=30μm, bottom=10μm. Adapted with permission from Macmillan Publishers Ltd: [Nature Methods], copyright (2010).

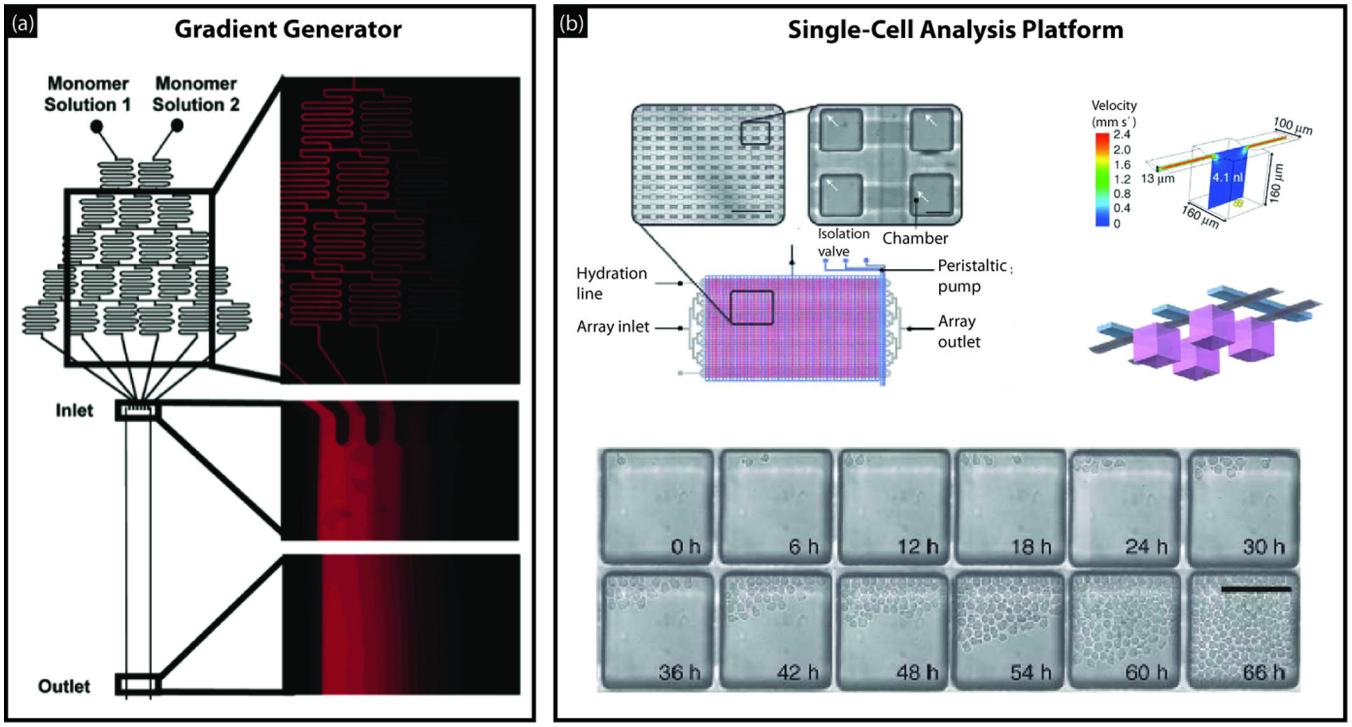

Figure 4.

Microfluidics used for dynamic biochemical presentation. (a) A ‘Christmas-tree’ microfluidic device used to produce soluble concentration gradients.[10] (Adapted with permission from American Chemical Society) (b) Microfluidic platform with nanoliter-sized chambers used for single-cell studies and temporally-varying exposure of cells to a cytokine.[74] Scale bar: bottom right=100µm. Adapted with permission from Macmillan Publishers Ltd: [Nature Methods], copyright (2011).

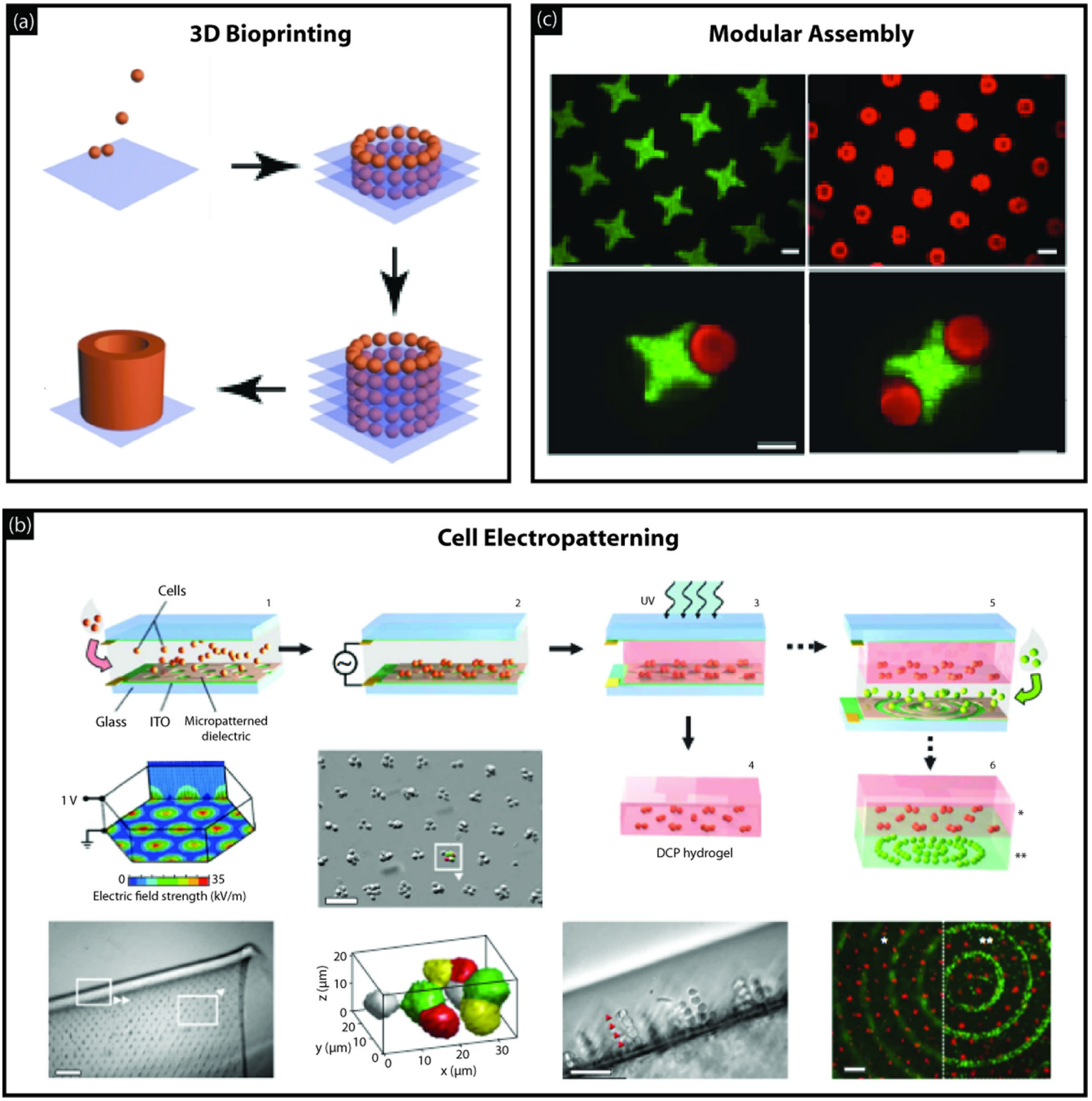

Figure 5.

Engineering methods used to construct tissue-like structures. (a) 3D bioprinting schematic: layer-by-layer deposition of cell aggregates.[127] (Adapted with permission from John Wiley and Sons) (b) Cell patterning and multicellular assembly by dielectrophoresis (DEP): DEP patterning of cells in prepolymer solution (1–2), photopolymerization (3), single- and multi-layer construct formation (4–6).[4] Scale bar: bottom right=100μm. Adapted by permission from Macmillan Publishers Ltd: [Nature Methods], copyright (2006). (c) Assembly of fluorescently-labeled hydrogels using lock-and-key shapes.[32] Scale bars: 200μm. Copyright (2008) National Academy of Sciences, USA.

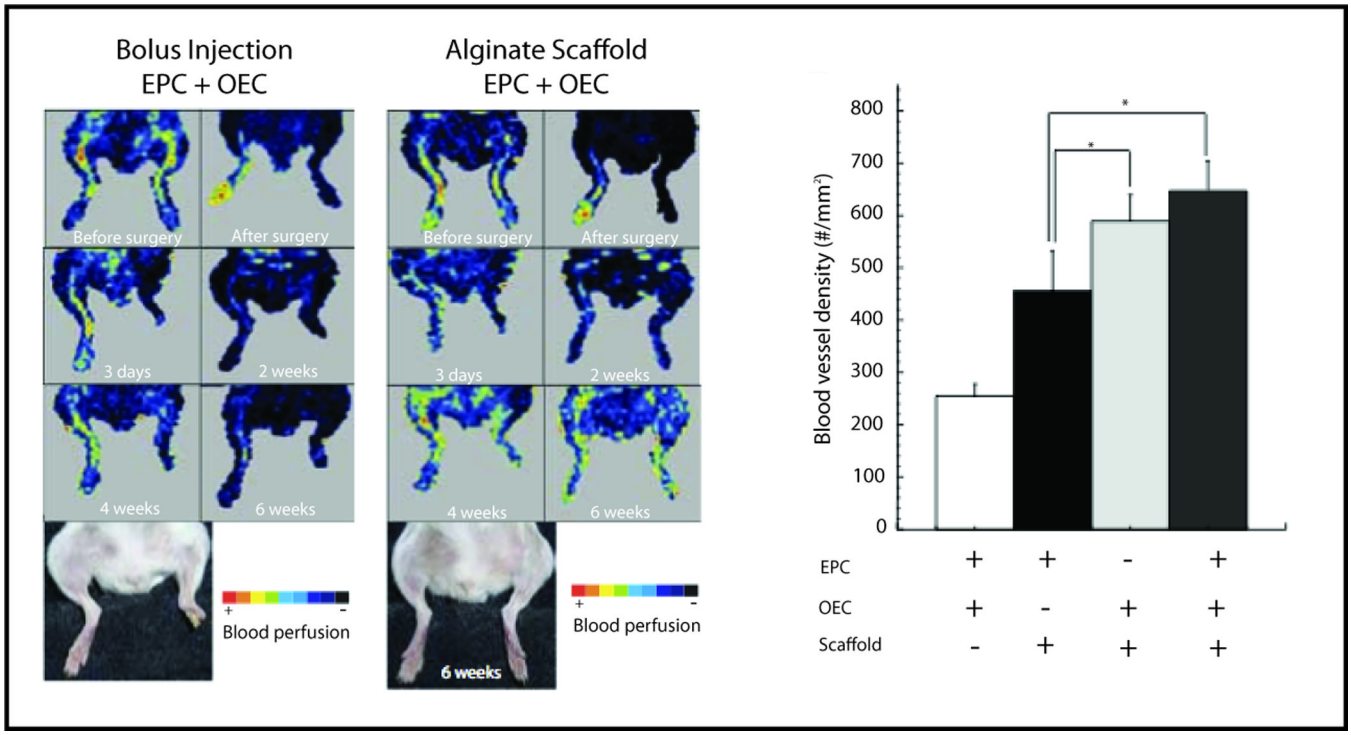

Figure 6.

Biomaterials as delivery vehicles for cells. Alginate scaffolds with RGD, VEGF, endothelial progenitor cells (EPC) and outgrowth endothelial cells (OEC) salvaged ischemic murine hind limbs as shown in gross and perfusion images.[120] Copyright (2008) National Academy of Sciences, USA.